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(12) # EUROPEAN PATENT SPECIFICATION

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(54) **Antibiotic clathrates.**

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(73) Proprietor: **WARNER-LAMBERT COMPANY**
201 Tabor Road
Morris Plains New Jersey 07950 (US)

(72) Inventor: **Ninger, Fred C.**
315 Harmon Cove Towers
Secaucus New Jersey 07094 (US)
Inventor: **Liao, Weichi**
84 Warwick Street
Iselin New Jersey 08830 (US)

(74) Representative: **Jones, Michael Raymond et al**
HASELTINE LAKE & CO. Hazlitt House 28
Southampton Buildings Chancery Lane
London WC2A 1AT (GB)

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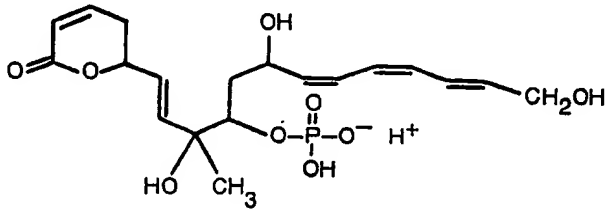
Description

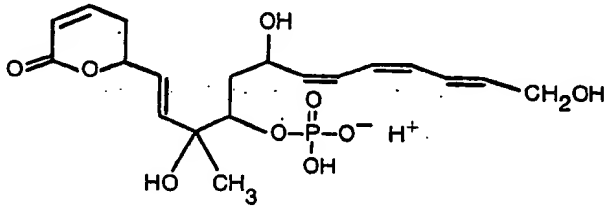
The compound designated phosphotrienin is a phosphorus-containing antibiotic produced by cultivating a specific strain of the genus *Streptomycetes* which produces the compound. It has antifungal and antitumor activity. The structural formula of the compound is:



This compound is also called pyranophosphate and is identified as CL 1565A. The compound CL 1565A, its characteristics and its production are described in United States Patent No. 4578383. The active compound is also useful in the form of its pharmaceutically acceptable salts.

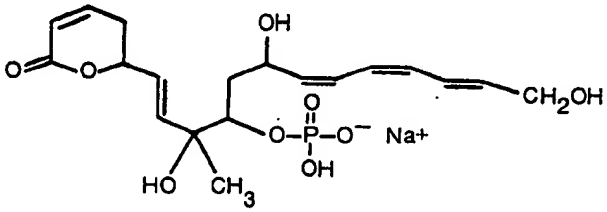
Owing to the presence of a molecular triene configuration, phosphotrienin undergoes rapid oxidative degradation in oxygen-containing environments. Its instability increases with increasing temperature. In view of its pronounced instability, its pharmacological and clinical evaluation appeared severely restricted unless a mechanism could be found to stabilize phosphotrienin to permit its use in formulating suitable dosage forms such as solutions and/or dry products such as tablets or capsules.

According to one aspect of the present invention, there is provided a cyclodextrin clathrate of the antibiotic compound phosphotrienin having the structural formula:



or of a pharmaceutically acceptable salt of phosphotrienin.

A preferred embodiment of this one aspect is a cyclodextrin clathrate of the sodium salt of phosphotrienin having the structural formula:



The present invention is based on the discovery that phosphotrienin can be stabilised against oxidative degradation by reacting it with various cyclodextrins to form inclusion compounds or clathrates. Specifically, it appears that the conjugated triene group therein is entrapped in the cavity of a cyclodextrin molecule and is thus protected by the shielding effect of the cyclodextrin from oxygen and/or other free radicals.

It has been found that, owing to the hydrophobic nature of the conjugated triene side chain, cyclodextrins have a higher affinity for this unsaturated side chain than for the other functional groups on the phosphotrienin molecule. In addition, the molecular dimensions estimated for the cavity of cyclodextrin and the corresponding triene group of phosphotrienin are compatible for the formation of clathrates.

Pharmaceutical preparations containing the clathrates of the present invention exhibit the antibiotic, antifungal, and antitumor properties of phosphotrienin, but do not exhibit its oxidative instability.

The phosphotrienin clathrate products in accordance with the present invention have several advantages over the parent compound from which they are derived. The steric protection of phosphotrienin through cyclodextrin clathrate formation provides, owing to the good stability of cyclodextrins, a shield for the oxidation-prone triene group. Additional protection for another functional group, namely the lactone ring, is possible when specific cyclodextrins having larger cavities are used.

Another of the present invention provides a pharmaceutical composition containing one or more of the clathrates of the first-mentioned aspect.

Phosphotrienin

The compound phosphotrienin (i.e. compound CL 1565A or pyranophosphate) is made by the cultivation of a specific strain of *Streptomycetes.*

The production and use of phosphotrienin and its salts are described in United States Patent No. 4578383. Examples of salts are sodium, potassium, magnesium, calcium, barium, aluminium, zinc, iron, ammonium and amine salts. The salts may contain from 1.0 to 2.0 equivalents of cation per equivalent of the parent free acid.

In general, the production and recovery of phosphotrienin is carried out using well-known techniques.

The cyclodextrins useful in complexing or clathrating the antibiotic compound phosphotrienin discussed above include most forms of cyclodextrin. One or more of the alpha-, beta-, gamma-, or delta-forms of cyclodextrin and various equivalents thereof can be employed. The *Merck Index,* 10th edition (1983), outlines the properties of several cyclodextrins on pages 389—90 (No. 2712).

The complexing reaction, or clathration, between phosphotrienin (or a salt thereof) and one or more cyclodextrins follows procedures which are generally well known and customarily used for the production of such inclusion compounds. Parameters such as temperature and pressure vary and depend upon solvents used and the physical characteristics of the reactants.

It is generally preferred to react aqueous or organic solvent solutions of phosphotrienin with an aqueous solution of the cyclodextrin. To ensure proper reaction, it is preferred that the organic solvent used be miscible with water.

The use of heat is usually not necessary.

The clathrates produced in solution are recovered by conventional techniques, e.g. solvent evaporation, and/or lyophilization. Other conventional separation and purification techniques can be used instead of, or in combination with, these techniques.

It should be noted that the clathrate complexes produced in accordance with the invention are useful not only *per se,* but also in combination with numerous diluents and excipients. For example, the use of excipients, carriers, diluents and other additives conventionally employed in the preparation of pharmaceutical dosage forms is contemplated.

The cyclodextrin clathrates produced herein are useful in a wide variety of pharmaceutical dosage forms. Phosphotrienin clathrates in aqueous solvents and/or other suitable solvents may be injected intravenously. Tablets, capsules, caplets, and other solid forms can be used to prepare oral dosage forms. Creams, lotions and other suitable forms may be formulated for topical administration.

The preferred process for producing the clathrate compounds is illustrated in the following examples.

Example 1

750 Milligrams of the monosodium salt of phosphotrienin were dissolved in 90 ml of water in which 0.8 g of alpha-cyclodextrin was dissolved. The final volume was then made up to 100 ml. About 43% of the phosphotrienin was estimated to be in the clathrate form of phosphotrienin with alpha-cyclodextrin in the solution after reaction.

Example 2

1.6 Grams of alpha-cyclodextrin were dissolved in 90 ml of water, and then 750 mg of the monosodium salt of phosphotrienin were added and dissolved. The final volume was made up to 100 ml. 75% of the phosphotrienin was estimated to be in the clathrate form with alpha-cyclodextrin in this solution after reaction.

Example 3

8.0 Grams of alpha-cyclodextrin were dissolved in 90 ml of water, and then 750 mg of the monosodium salt of phosphotrienin were added and dissolved. The final volume was made up to 100 ml. The active compound, phosphotrienin, was estimated at 95% as the clathrate with alpha-cyclodextrin in this solution after reaction.

Example 4

Solutions obtained in Examples 1, 2 and 3 were lyophilized. The percentage of active compound in the clathrate form was estimated to be 50%, 100% and 100% respectively in the lyophilized powder.

Examples 5, 6, 7

Instead of using water as the solvent, as given in Examples 1, 2 and 3, a 60% aqueous ethanol was used as an alternate solvent. The application of vacuum and subsequent drying at 34—40°C, or the application of lyophilization, produced a white powder of alpha-cyclodextrin clathrate of phosphotrienin.

Example 8

1.88 Grams of beta-cyclodextrin were dissolved in 200 ml of water; 750 mg of the monosodium salt of

phosphotrienin were added and dissolved. The solution was lyophilized to produce a powder of the beta-cyclodextrin clathrate of phosphotrienin.

Example 9

750 Milligrams of the monosodium salt of phosphotrienin were dissolved in 100 ml of water containing 2.15 g of gamma-cyclodextrin. The solution was lyophilized to produce a powder of the gamma-cyclodextrin clathrate of phosphotrienin.

Example 10

750 Milligrams of the monosodium salt of phosphtrienin were dissolved in 100 ml of water containing 2.42 g of delta-cyclodextrin. The resulting solution was lyophilized to produce a powder of delta-cyclodextrin clathrate of phosphotrienin.

The equilibrium constant for the clathrate formation between phosphotrienin and alpha-cyclodextrin was measured in an aqueous buffer solution at pH 6. This constant was employed to estimate the extent of clathrate formation in Examples 1, 2, 3 and 4.

Thermal stability tests confirmed the excellent stabilities of the clathrate compounds compared with original phosphotrienin (or its salt). The results of the thermal stability test via HPLC assay of cyclodextrin clathrate of phosphotrienin is shown below:

Lyophilized Powder	% Remaining (at 25°C)
phosphotrienin	70% (after 5 weeks)
Alpha-cyclodextrin clathrate of CL 1565A	100% (after 4 weeks)

The following examples illustrate the preferred methods of using the clathrate compounds of this invention in pharmaceutical dosage forms.

Example 11

Phosphotrienin alpha-cyclodextrin Solutions for Injection

Solutions, as prepared in Examples 1, 2 and 3, were prepared and divided into ampoules under sterile manufacturing conditions such that each ampoule contained the required dose for therapeutic indications. Each ampoule was flushed with nitrogen gas and sealed.

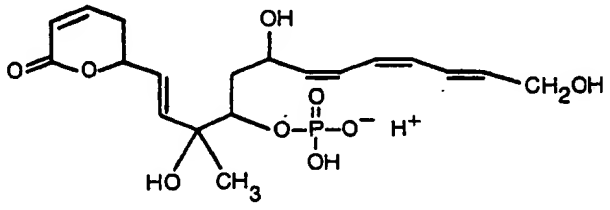
Example 12

Phosphotrienin alpha-cyclodextrin Lyophilized Powder for Injection

Solutions, as prepared in Examples 1, 2 and 3, were prepared and divided into vials under sterile manufacturing conditions such that each vial contained a suitable dose for therapeutic activity. Lyophilization was carried out to remove the water and obtain a white powder. Each vial was capped and sealed. This was useful as an injection with the addition of normal saline solution for injection.

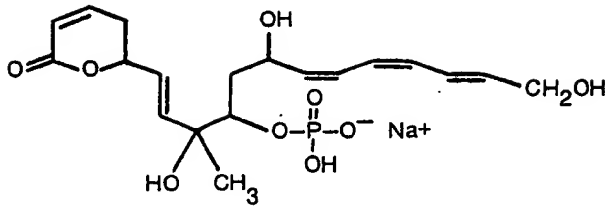
Claims

1. A cyclodextrin clathrate of the antibiotic compound phosphotrienin having the structural formula:



or of a pharmaceutically acceptable salt of phosphotrienin.

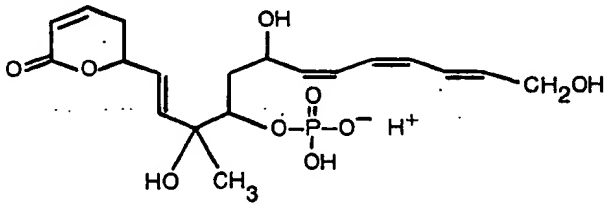
2. A cyclodextrin clathrate of the sodium salf of phosphotrienin having the structural formula:



3. The alpha-cyclodextrin clathrate of the phosphotrienin having the formula shown in Claim 1 or 2.
4. The beta-cyclodextrin clathrate of the phosphotrienin having the formula shown in Claim 1 or 2.
5. The gamma-cyclodextrin clathrate of the phosphotrienin having the formula shown in Claim 1 or 2.
6. The delta-cyclodextrin clathrate of the compound phosphotrienin having the formula shown in Claim 1 or 2.
7. A pharmaceutical composition containing one or more of the clathrates according to Claim 1 or 2.
8. A pharmaceutical composition containing one or more of the clathrates according to Claim 3.
9. A pharmaceutical composition containing the clathrate according to Claim 4.
10. A pharmaceutical composition containing the clathrate according to Claim 5.
11. A pharmaceutical composition containing the clathrate according to Claim 6.

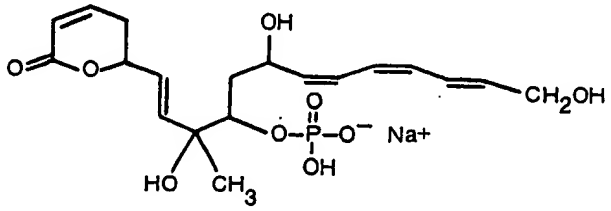
Patentansprüche

1. Ein Zyklodextrinklathrat der antibiotischen Verbindung Phosphotrienin mit nachstehender Strukturformel:



oder eines pharmazeutisch akzeptablen Salzes des Phosphotrienin.

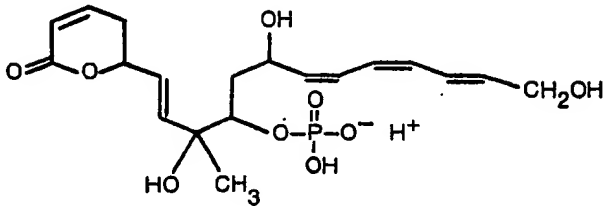
2. Ein Zyklodextrinklathrat des Natriumsalzes des Phosphotrienin mit nachstehender Strukturformel:



3. Das Alpha-Zyklodextrinklathrat des Phosphotrienin mit der in den Patentansprüchen 1 oder 2 dargestellten Formel.
4. Das Beta-Zyklodextrinklathrat des Phosphotrienin mit der in den Patentansprüchen 1 oder 2 dargestellten Formel.
5. Das Gamma-Zyklodextrinklathrat des Phosphotrienin mit der in den Patentansprüchen 1 oder 2 dargestellten Formel.
6. Das Delta-Zyklodextrinklathrat der Phosphotrienin-Verbindung mit der in den Patentansprüchen 1 oder 2 dargestellten Formel.
7. Eine pharmazeutische Verbindung, die eine oder mehrere der Klathrate nach Patentanspruch 1 oder 2 enthält.
8. Eine pharmazeutische Verbindung, die eine oder mehrere der Klathrate nach Patentanspruch 3 enthält.
9. Eine pharmazeutische Verbindung, die das Klathrat nach Patentanspruch 4 enthält.
10. Eine pharmazeutische Verbindung, die das Klathrat nach Patentanspruch 5 enthält.
11. Eine pharmazeutische Verbindung, die das Klathrat nach Patentanspruch 6 enthält.

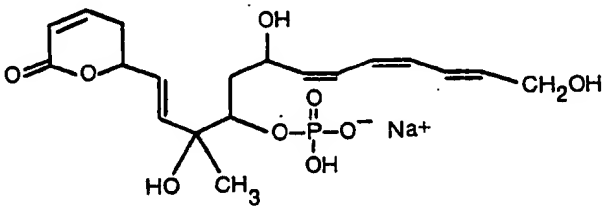
Revendications

1. Un clathrate cyclodextrinique du composé antibiotique phosphotriènine ayant la formula développée:



ou un sel de la phosphotriènine acceptable sur le plan pharmaceutique.

2. Clathrate cyclodextrinique du sel de sodium de la phosphotriènine ayant la formule développée:



3. Clathrate alpha-cyclodextrinique de la phosphotriènine ayant la formule indiquée dans les revendications 1 ou 2.

4. Clathrate bêta-cyclodextrinique de la phosphotriènine ayant la formule indiquée dans les revendications 1 ou 2.

5. Clathrate gamma-cyclodextrinique de la phosphotriènine ayant la formule indiquée dans les revendications 1 ou 2.

6. Clathrate delta-cyclodextrinique de la phosphotriènine ayant la formule indiquée dans les revendications 1 ou 2.

7. Composition pharmaceutique contenant un ou plusieurs des clathrates selon les revendications 1 ou 2.

8. Composition pharmaceutique contenant un ou plusieurs des clathrates selon la revendication 3.

9. Composition pharmaceutique contenant le clathrate selon la revendication 4.

10. Composition pharmaceutique contenant le clathrate selon la revendication 5.

11. Composition pharmaceutique contenant le clathrate selon la revendication 6.